

Role of Uranium in Mutagenesis of the *PKDI* Gene in Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) affects over 500,000 people in the U.S. and is responsible for 8-10% of all chronic renal replacement therapy. Mutations in the *PKDI* gene are responsible for 95% of cases. This gene has the longest polypurine polypyrimidine tract in the human genome, and this tract has multiple segments that are capable of forming triplex DNA structures that can block the replication fork in primer extension reactions. Replication fork blockade requires recombinational repair, and such error-prone repair could result in mutation of the functional gene. Such a loss of function at the locus (two-hit hypothesis) leads to disease. Triplex structures, such as those that can form from the sequences in the *PKDI* gene, are more easily formed in the presence of metal ions. We hypothesized that depleted uranium ions could facilitate triplex formation and augment replication fork blockade. This hypothesis was tested directly using basic biochemical techniques, as well as from a population-based approach looking at the severity of ADPKD from an area with a population with a greater depleted uranium exposure.

In the laboratory component of this project, the SV40 plasmid replication system was used. A triplex forming sequence from the *PKDI* gene known to block replication was cloned into the plasmid pZ189. This plasmid initiates bidirectional replication using the SV40 large T antigen. Replication reaction products were run on agarose gels and visualized by southern transfer and chemiluminescent detection. While successful, the chemiluminescent detection system was time-consuming with inadequate reproducibility. Replication in the presence of radioactive dCTP proved to be highly sensitive and reproducible. The triplex forming sequence from the *PKDI* gene demonstrated a strong replication fork blocking effect. No replication effect could be identified at 20 ng uranium ion, but significant replication suppression was seen using 1 µg per replication reaction. Titration of the uranium replication effects is underway to determine if depleted uranium synergizes the replication blocking activity of the triplex forming sequence.

For the clinical component of this project, we researched preliminary data looking at susceptibility to ADPKD in association with uranium exposure among individuals in the Fernald Medical Monitoring Program (FMMP). The FMMP is a surveillance program for about 9500 people who lived near the uranium processing plant at Fernald, Ohio. Based on general population prevalence, there should be approximately ten people with ADPKD in the FMMP population. If depleted uranium has the hypothesized replication effects, then the exposed individuals would be expected to have an accelerated disease process given the two-hit hypothesis. We reviewed medical charts of FMMP participants with reported diagnosis of ADPKD and interviewed various family members. With this information, we developed pedigrees for two families where ADPKD has clustered. Members of these families with ADPKD who lived in the Fernald domain exposure have relatively young age of onset of their disease, as young as eight years. Further details about age of onset, disease severity, and

drinking water source (public water; home well or cistern) in members of these families living in and out of the exposure domain are being obtained.